Universidade de Aveiro Departamento de Biologia

2015

André Pedro do Couto

Efeitos locais e paisagísticos na diversidade de anfíbios no nordeste de Portugal.

Local and landscape effects on the amphibian diversity in northeastern Portugal.

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Ilustração da capa: *Salamandra salamandra*.

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica do Prof. Doutor Carlos Manuel Martins Santos Fonseca, Professor Auxiliar com Agregação do Departamento de Biologia, e coorientação científica do Doutor Eduardo Manuel Silva Loureiro Alves Ferreira, Professor Auxiliar Convidado do Departamento de Biologia, e da Doutora Rita Maria Tinoco da Silva Torres, Investigadora Pós-Doutoramento do Centro de Estudos do Ambiente e do Mar, da Universidade de Aveiro.

A um Reino Maravilhoso, de natureza e tradição,

O Júri

Presidente

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Coordenador do Gabinete de Comunicação de Ciência do Centro Interdisciplinar de Investigação Marinha e Ambiental.

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Palavras-chave Mediterrânico, agricultura tradicional, hidroperíodo, charcos, conservação, modelação de habitat.

Resumo

Estes estudo teve como objetivo determinar quais os fatores que influenciam a diversidade de anfíbios num ecossistema Mediterrânico caraterizado por um mosaico de áreas naturais e uso tradicional do solo, e gerar informação prática para a conservação e gestão de habitats. Foram amostrados 60 charcos temporários e permanentes no nordeste de Portugal, determinando a sua riqueza específica, e composição da comunidade de anfíbios. As relações entre estas medidas e vários fatores ambientais foram analisadas dentro de escalas espaciais locais (*buffers* de 5 e 50 m) e paisagísticas (*buffer* de 500 m). Estes fatores descreveram (1) caraterísticas do charco e cobertura do terreno envolvente; (2) heterogenidade do habitat; (3) uso tradicional do solo e densidade de estradas. Tanto a riqueza específica como a composição da comunidade foram maioritariamente influenciadas por fatores locais. A riqueza específica esteve positivamente correlacionada com o hidroperíodo do charco, e com a heterogenidade de habitats e matos em 5 m; e negativamente correlacionada com areas de solo nú e densidade de estradas em 50 m. A única variável paisagística que influenciou a riqueza específica foi a coberterura de coníferas em 500 m, com uma associação positiva. O hidroperíodo foi também o fator mais determinante na composição das comunidades, juntamente com a vegetação aquática, e várias variáveis de cobertura do terreno. Os terrenos de agricultura tradicional não tiveram, de uma forma geral, impacto na diversidade de anfíbios, podendo ter contríbuido para o aumento da heterogenidade ambiental, e disponibilizando locais de reprodução.Os resultados obtidos reforçam a ideia que a biodiversidade pode coexistir com a agricultura tradicional, e que esta pode ter um papel importante na conservação. Propõe-se que em cenários semelhantes, as medidas de conservação e gestão do habitat de anfíbios foquem o meio aquático e o habitat local envolvente.

Keywords Mediterranean, traditional agriculture, hydroperiod, wetlands, conservation, habitat modelling.

Abstract This study aimed to determine which factors influence amphibian diversity in a Mediterranean ecosystem characterized by a mosaic of natural areas and traditional land use, providing practical information for amphibian conservation and habitat management. We sampled 60 temporary and permanent ponds in northeastern Portugal, determining their amphibian species richness and assemblage composition. Relationships between these measures and environmental factors were analyzed within local (5 and 50 m buffers) and landscape scale (500 m buffer). These factors described (1) wetland characteristics and surrounding land cover; (2) habitat heterogeneity; (3) traditional farmland and local road density. Both species richness and assemblage composition were mainly influenced by local factors. Species richness was positively correlated with pond hydroperiod, habitat heterogeneity and scrub within 5 m; and negatively correlated with bare ground areas and road density within 50 m. Coniferous forest was the only landscape variable explaining species richness, with a positive association. Hydroperiod was also the most important factor determining assemblage composition, along with aquatic vegetation, and several land cover variables. Traditional farmland had no impact on amphibian diversity overall, and might provide habitat heterogeneity and breeding sites for amphibians. Our results support the view that biodiversity can coexist with traditional farming activities and that these practices might have important conservation value. We propose that in similar scenarios, amphibian conservation and habitat management should focus on wetlands and local habitat.

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CAPÍTULO 1: INTRODUÇÃO GERAL

1.1. Diversidade e distribuição global dos anfíbios

Atualmente são conhecidas 7 455 espécies de anfíbios (Frost, 2015), um número que estará ainda distante da realidade, avaliando atual ritmo de descoberta de novas espécies (Köhler et al., 2005). Estes organismos estão distríbuidos por quase todo o planeta, com exceção das regiões mais frias e secas, e algumas ilhas remotas (Stuart et al., 2008). Taxonomicamente, constituem a classe Amphibia, que se divide nos clados Gymnophiona (ápodes, restritos aos trópicos e sub-trópicos), Caudata (salamandras e tritões), e Anura (sapos, rãs, e relas) (Vitt & Caldwell, 2014). É nos biomas tropicais que este grupo apresenta maior riqueza específica, principalmente porque grande parte dos 6 554 anuros conhecidos se encontram nos trópicos (Frost, 2015). Por outro lado, a maioria dos urodelos (695 espécies) habita nas regiões temperadas do Hemisfério Norte (Stuart et al., 2008).

1.2. O papel dos anfíbios nos ecossistemas

Apesar de serem animais discretos, devido ao tamanho reduzido, hábitos maioritariamente noturnos, e períodos de atividade curtos e bastante sazonais, os anfíbios são frequentemente organismos abundantes e parte essencial dos ecossistemas que habitam (Wells, 2007). Ilustrativo desta importância é o estudo realizado por Burton & Likens (1975) numa floresta temperada da América do Norte, onde a biomassa estimada de salamandras foi duas vezes mais alta que a de aves e semelhante à de mamíferos, e a densidade de indivíduos foi maior que a de ambos os grupos. Recentemente, Semlitsch et al. (2014) registaram valores muito superiores para os mesmos parâmetros, salientado a importância dos anfíbios nas teias alimentares e no fluxo de energia e nutrientes. Enquanto presas, são consumidos por uma grande variedade de animais invertebrados e vertebrados (Duellman & Trueb, 1994). Não só compõem uma parte considerável da dieta de vários répteis, aves, e mamíferos, como são a única fonte de alimento para alguns predadores especializados (Toledo et al., 2007). Por outro lado, no papel de consumidores podem alimentar-se de matéria vegetal (no caso das larvas de anuros), de

invertebrados terrestres e aquáticos, e até mesmo de outros vertebrados (Duellman, 2005; Altig et al., 2007; Do Couto & Menin, 2014). Frequentemente, o impacto destas relações transcende as espécies envolvidas; por exemplo, a estrutura da comunidade de algas, ciclo de azoto, e composição do sedimento de corpos hídricos pode ser drasticamente alterada em função da presença ou ausência de girinos (Whiles et al., 2006). Por outro lado, a predação por parte de adultos e larvas carnívoras exerce controlo de inverterbrados aquáticos, terrestres, e aéreos (Wyman, 1998; Beard et al., 2003; Rubbo et al., 2011). Estes processos são importantes não só para a estabilidade dos ecossistemas, mas também trazem benefícios diretos para a sociedade e economia, se considerarmos o papel dos invertebrados e dos insectos em particular, enquanto pragas agrícolas e vectores de doenças (Klepzig et al., 2009; Rubbo et al., 2011).

1.3. Relações com o habitat, ameaças e conservação

O ciclo de vida dos anfíbios, salvo raras exceções, é caraterizado pela dependência tanto de habitats terrestres como aquáticos. Durante a época de reprodução, a maioria das espécies deposita os ovos ou as larvas em meios aquáticos diversos, desde pequenas acumulações de água da chuva em plantas até lagos de grande dimensão, onde se desenvolvem até completarem a metamorfose (Pough et al., 2004). Enquanto adultos, alguns anfíbios mantêm hábitos totalmente aquáticos (ex: Pipidae), mas na maior parte das espécies ocorre uma transição para o meio terrestre (Vitt & Caldwell, 2014). Esta associação à água e à terra, juntamente com caraterísticas morfofisiológicas como a pequena dimensão corporal, metabolismo ectotérmico, e pele permeável, faz com que sejam um grupo particularmente sensível ao meio que os rodeia (Wells, 2007).

Nas últimas décadas, tem-se observado um declínio global nas populações de anfíbios, sendo atualmente um dos grupos animais mais ameçados. Cerca de um terço das espécies conhecidas estão ameaçadas e classificadas pela *International Union for Conservation of Nature* (IUCN) como “Criticamente em Perigo” (8%), “Em Perigo” (13%) ou “Vulnerável” (11%) (Stuart et al., 2008). Igualmente preocupante é o facto de não existir informação suficiente para atribuir um estatuto de conservação a 23% das espécies, mas sendo grande parte destas raras e com distribuição limitada, é provável que se enquadrem nas categorias já referidas. Adicionalmente, nas restantes espécies (44%) observa-se também um padrão geral menos acentuado de decréscimo das

populações. A principal causa deste declínio é, acima de tudo, a destruição e modificação do habitat, mas também a poluição, a introdução de espécies exóticas, e a propagação de doenças como a quitridiomicose *Batrachochytrium dendrobatidis* (Stuart, 2004). Estas ameaças estão frequentemente associadas com a expansão e intensificação da agricultura, o desenvolvimento urbano, e com alterações climáticas, afetando tanto habitats aquáticos como terrestres (Pounds et al., 2006; Hamer & McDonnell, 2008; Mann et al., 2009).

Whiles et al. (2006) refere que "*Devido às diferenças funcionais e de habitat entre larvas e adultos na maioria dos anfíbios, a perda de uma espécie é na realidade equivalente à perda de duas*", o que exemplifica a gravidade do declínio destes animais e a importância da sua conservação. Neste sentido, várias medidas de conservação têm sido postas em prática nos últimos anos, desde proteção, melhoramento e criação de habitats (Pechmann et al., 2001; Hamer et al., 2002); controlo de espécies invasoras (Kats & Ferrer, 2003; Amaral & Rebelo, 2012;); reprodução em cativeiro e reintrodução de espécies ameaçadas (Zippel et al., 2011), para dar alguns exemplos. O sucesso, aplicabilidade, e custo destas medidas varia consoante a situação, no entanto, a longo prazo passará certamente pelo estudo aprofundado dos processos envolvidos, por decisões dos poderes políticos e administrativos, e pela sensibilização, educação, e envolvimento da população geral (Stuart et al., 2008).

1.4. Os anfíbios na Europa, no Mediterrâneo, e na Península Ibérica

No continente europeu estão presentes 73 espécies de anfíbios (Sillero et al., 2014), que se encontram, na sua maioria, distribuídas pelo oeste centro e sul do continente. Neste cenário destaca-se a importância da bacia do Mediterrânico, classificado como um *hotspot* de biodiversidade pela IUCN (Cuttelod et al., 2008). Esta região que engloba partes da Europa, Norte de África e Médio Oriente, apresenta um clima distinto, caraterizado por verões quentes e secos, e invernos amenos (Cuttelod et al., 2008; Filipe et al., 2012). A diversidade atual de flora e fauna foi moldada não só por estes aspetos, mas também pela elevada presença humana nos últimos séculos (Cuttelod et al., 2008). Aqui ocorrem 106 espécies de anfíbios, dois terços das quais são endémicas (Cox et al.,2006). Ao longo deste trabalho, as menções da região Mediterrânica serão relativas à porção europeia, uma vez que a anfíbiofauna desta área é mais semelhante à que está presente no resto da Europa do que a dos restantes continentes. Neste cenário destaca-se a importância da Península Ibérica, que terá sido

um dos refúgios glaciares dos anfíbios europeus, sendo a sua diversidade atual um reflexo disso mesmo (Weiss & Ferrand, 2007). Porém, tal como acontece no resto da região Mediterrânica e na Europa em geral, as principais ameaças globais aos anfíbios (e à biodiversidade em geral) estão aqui bem presentes. Vários estudos têm demonstrado as consequências da destruição dos habitats e da poluição, particularmente no que diz respeito aos efeitos da agricultura intensiva (Beja & Alcazar, 2003; García-Muñoz et al., 2010; Ferreira & Beja, 2013). A introdução de espécies exóticas, como peixes ou crustáceos predadores tem causado o decréscimo e mesmo o colapso total de algumas comunidades de anfíbios (Bosch et al., 2006; Cruz et al., 2008). Finalmente, vários surtos de quitridiomicose têm sido reportados nas regiões montanhosas da Península, causando mortalidade em massa, particularmente no sapo-parteiro-comum (*Alytes obstetricans*) (Bosch et al., 2001; Rosa et al., 2013).

1.5. O Parque Natural do Montesinho e a serra da Nogueira

O Parque Natural do Montesinho (PNM) é uma área protegida no nordeste transmontano, mais precisamente nos concelhos de Bragança e Vinhais, distrito de Bragança, Portugal. Criado em 1979, abrange uma área de 74 800 ha limitada a norte e a este por Espanha. O clima é Mediterrânico, mas apresenta influências Atlânticas e Continentais, vindas do norte e do centro da Península Ibérica, respectivamente, dando origem ao que é regionalmente conhecido como "*Terra Fria*". Estas condições resultam numa variação anual caracterizada por invernos longos e frios e verões quentes e secos, que marcam profundamente os valores naturais e culturais da região (Castro et al., 2010). Nesta área (descrita com mais detalhe posteriormente) é possível observar uma grande diversidade de paisagens naturais, desde a alta montanha da serra do Montesinho, aos matagais e pinhais da serra da Lombada, até aos carvalhais mais caraterísticos da região sul do Parque, que se estendem pela serra da Nogueira. Esta última, situada entre os concelhos de Vinhais, Bragança e Macedo de Cavaleiros, está juntamente com o PNM inserida na Zona de Proteção Especial (ZPE) Montesinho/Nogueira, ao abrigo do programa Rede Natura 2000. Para além dos habitats naturais, a paisagem da região é também marcada por atividades agrícolas de caráter maioritariamente extensivo e tradicional, particularmente a produção de cereais e de castanha. O pastoreio é também uma atividade importante, frequentemente dependente de lameiros, prados semi-naturais normalmente associados a corpos hídricos (Castro et al., 2010). A riqueza faunística é

também de salientar, particularmente a presença de espécies emblemáticas e de elevado valor conservacionista como a víbora-cornuda (*Vipera latastei*), a cegonha-negra (*Ciconia nigra*), e o lobo-ibérico (*Canis lupus signatus*) (Santos et al., 2007; Loureiro et al., 2008; Svensson et al., 2008).

A diversidade de anfíbios do PNM foi inicialmente conhecida através de alguns estudos de distribuição de herpetofauna a nível nacional (Crespo & Oliveira, 1989; Castrol et al. (1989) e Malkmus (1995), citados por Teixeira, 1997) e posteriormente com o levantamento de anfíbios e répteis do PNM no final da década de 90, realizado por José Teixeira (1997, 1998, 1999). Mais recentemente foi publicado o Atlas dos Anfíbios e Répteis de Portugal (Loureiro et al., 2008), de onde foi possível obter também a informação relevante à Serra da Nogueira. Estas áreas contam com a presença de 13 das 19 espécies de anfíbios portugueses: salamandra-de-costelas-salientes (*Pleurodeles walt),* salamandra-de-pintas-amarelas *(Salamandra salamandra),* tritão-de-ventre-laranja *(Lissotriton boscai),* tritão-marmoreado *(Triturus mamoratus),* sapo-de-unha-negra *(Pelobates cultripes),* sapo-parteiro-comum *(Alytes obstetricans),* sapo-parteiro-ibérico *(Alytes cisternasii),* rã-de-focinho-pontiagudo *(Discoglossus galganoi),* sapo-corredor *(Epidalea calamita),* sapo-comum *(Bufo spinosus),* rela-comum *(Hyla molleri),* rã-ibérica *(Rana iberica) e* rã-verde *(Pelophylax perezi).* Representa assim uma das regiões com maior riqueza específica de anfíbios em toda a Península Ibérica (Araújo et al., 2007). Apesar disso, a informação e quantidade de estudos científicos relativamente aos anfíbios da região é notoriamente reduzida: para além dos trabalhos já mencionados existe, do que foi possível averiguar, apenas um trabalho relativo à filogeografia de *A. obstetricans*, em que parte das amostras foram recolhidas no PNM (Gonçalves et al., 2015).

1.6. Objetivos gerais

Este trabalho teve como principal objetivo estudar as relações entre os anfíbios e o meio envolvente, tendo em conta diferentes escalas espaciais, processos naturais, e a influência antropogénica. Os resultados obtidos pretendem beneficiar não só o estudo da ecologia, mas também gerar informação prática para conservação destes animais em habitats Mediterrânicos. Adicionalmente, considerou-se importante contribuir para o conhecimento da fauna de anfíbios da regiões nordestina e para a herpetologia ibérica.

CAPÍTULO 2: "LOCAL AND LANDSCAPE EFFECTS ON THE AMPHIBIAN DIVERSITY IN NORTHEASTERN PORTUGAL."

2.1. Introduction

Recent population declines in amphibian species in Europe have been mainly caused by habitat destruction and modification (Stuart et al., 2008). Due to a combination of morphologic and physiologic traits, this group of animals is highly sensitive to environmental changes (Wells, 2007). Most European amphibians rely on wetlands, ponds in particular, for reproduction; therefore, a large part of the conservation efforts aiming amphibian populations is centered on these waterbodies and surrounding areas (Oertli et al., 2009). Despite considerable interspecies variation concerning migratory movements, most amphibians tend to remain close to breeding sites (Semlitsch, 2008). For this reason, species presence is often determined by local habitat factors. These can be related both to pond characteristics, such as hydroperiod, aquatic vegetation, or presence of predators (Semlitsch, 2000; Hartel et al., 2007); and riparian or surrounding habitats, concerning vegetation structure, or land use (Werner & Glennemeier, 1999; Semlitsch, 2000). Nevertheless, surrounding habitats at a landscape scale can also influence amphibian diversity, especially in human-modified areas (Ficetola & De Bernardi, 2004; Hartel et al., 2010; De la Montaña et al., 2011). Human-induced landscape modifications might not only change the proportion of specific land cover in a given area, but also influence habitat heterogeneity (Tews et al., 2004). Habitat heterogeneity is often considered one of the major factors explaining biodiversity, acting at varied spatial scales (Pianka, 1966). Studies have shown positive effects of local habitat heterogeneity on amphibian richness (Canova & Marchesi, 2007; Vasconcelos et al., 2009); however, while this was also observed at wider spatial scales, associations with particular habitats appeared to be more relevant (Atauri & de Lucio, 2001; Moreno-Rueda & Pizarro, 2007).

Asphalt roads are another important main source of anthropogenic impact on amphibians biodiversity (Trombulak & Frissel, 2000). Even rural local roads with low traffic intensity can act as barriers for migrating or dispersing individuals as a consequence of roadkill (Garcia-Gonzalez et al., 2012). Roads and traffic might also cause several types of alterations in the surrounding environment such as increased pollution, modification of

plant communities, and noise or visual disturbance (see Coffin, 2007), which in turn can directly impact breeding wetlands.

In the European continent, the Mediterranean Basin is considered a hotspot for plant and vertebrate biodiversity, which is also reflected on the high amphibian species richness in this region (Cox et al., 2006). Several recent studies have highlighted the importance of Mediterranean wetlands for amphibian conservation (Gómez-Rodríguez et al., 2009; D'Amen et al., 2011; Ribeiro et al., 2011; Ferreira & Beja, 2013) which are among the most threatened habitats in the world (Cuttelod et al., 2008). Agricultural intensification is one of main causes of habitat destruction and modification in this region, and several authors have demonstrated its negative effects on amphibian populations (Beja & Alcazar, 2003; Ficetola & De Bernardi, 2004; García-Muñoz et al., 2010; Ferreira & Beja, 2013). Conversely, the Mediterranean region is also undergoing a widespread abandonment of agricultural lands, however this seems to affect mostly areas where agricultural practices are still largely traditional (Keenleyside & Tucker, 2010). Traditional farmland is usually characterized by small scale rotative cultures (fallow system) with low use of chemicals and semi-managed pastures that allow natural features and habitat diversity (Baldock et al., 1994). While the decline of this kind of land use is frequently considered a problem for biodiversity conservation (European Environment Agency, 2004), this view has been contested by Navarro & Pereira (2012), stating that land abandonment could constitute an opportunity for European wilderness to recover some of its past distribution range. Several authors have reported the positive effects of traditional farming practices on amphibian diversity (*e.g.*: Fujioka & Lane, 1997; Joly et al., 2001; Johansson et al., 2005). However, these studies often compare extensive and intensive agricultural systems and, in the Mediterranean context, information relative to contact zones between natural habitats and traditional farmland is still relatively scarce (but see: Crochet et al., 2004; De la Montaña et al., 2011).

The present study took place in the northern edge of the Mediterranean, specifically northeastern Portugal, where large parts of forest and other natural habitats remain preserved (Castro et al., 2010). Agricultural practices are still largely traditional but are currently declining, due to land abandonment. Within local and landscape scales, we analyzed the relationship of amphibian species richness and assemblage composition with: (1) wetland characteristics and surrounding land cover; (2) habitat heterogeneity; (3) traditional farmland and local road density. Our aim was to determine which factors influence amphibian diversity in a Mediterrenean ecosystem characterized by natural

areas and traditional land use, and to provide practical information for amphibian conservation and habitat management.

2.2. Materials & Methods

2.2.1. Study area

This study was carried out in Montesinho Natural Park (6°48'37.44"W, 41°54'3.27"N) and Serra da Nogueira (6°53'22.60"W, 41°47'5.10"N). Both areas are part European Union's Natura 2000 network, covering an area of 108 011 ha in northeastern Portugal (Figure 1). Altitudinal values range from 436 to 1487 m, with most areas situated between 700 to 900 m. The climate is Mediterranean with Atlantic influence, from the Spanish high mountain ranges to the north, and Continental influence from the inner Iberian Pensinsula to the east (Castro et al., 2010). Mean temperature varies between 4 ºC in the coldest month and 21 ºC in the warmest month with mean annual precipitation around 770mm (IPMA, 2015). The landscape is defined by a mosaic of deciduous forest (*Quercus pyrenaica, Castanea sativa*, *Quercus rotundifolia),* conifers *(Pinus pinaster, Pinus sylvestris, Pseudotsuga menziesii),* scrubland (*Erica* spp., *Cistus ladanifer, Cytisus spp.,Genista spp.)* and traditional farmland consisting mainly of extensive cereal fields and orchards. Upland semi-natural pastures associated with the various streams and rivers that cross the region are bordered by hedgerows dominated by *Fraxinus angustifolia, Alnus glutinosa* and *Populus nigra*. Temporary ponds occur naturally as a direct result of rain, or due to flooding along river banks. Most permanent ponds were dug to serve either as reservoirs for firefighting during the hot summer months, or as water sources for agriculture and livestock. Human presence is low (9.5 people/km^2) and restricted to small villages. The area is crossed by a number of national and local roads. Thirteen species of amphibians are known to occur in this area: *Pleurodeles walt, Salamandra salamandra, Lissotriton boscai, Triturus marmoratus, Pelobates cultripes, Alytes obstetricans, Alytes cisternasii, Discoglossus galganoi, Epidalea calamita, Bufo spinosus, Hyla molleri, Rana iberica* and *Pelophylax perezi*, which correspond to about half of the Iberian Peninsula amphibian diversity (Teixeira, 1997; Pleguezuelos et al., 2002; Loureiro et al., 2008).

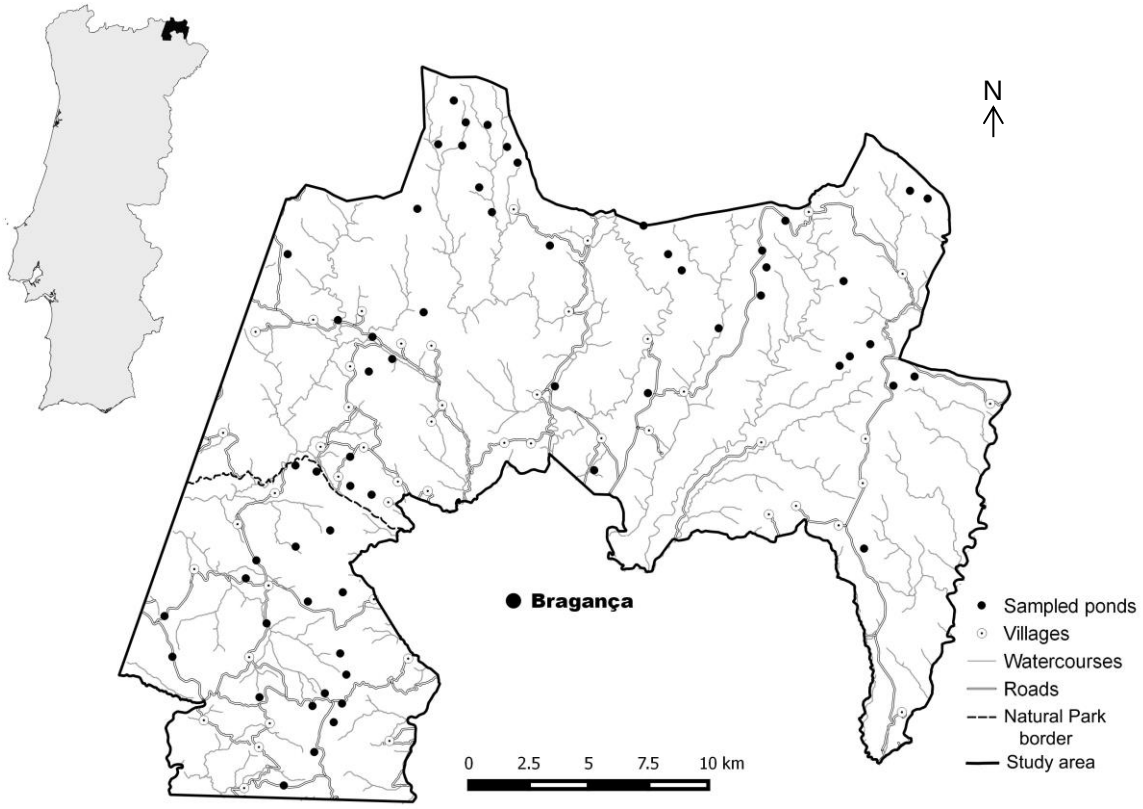

Figure 1 – The study area, located in Montesinho Natural Park and Serra da Nogueira, northeastern Portugal. Bragança is the district capital and nearest city.

2.2.2. Field surveys

Between January and October 2015, 60 ponds were sampled bi-monthly. The surveys were done at night, by active search with head torches, along wetland margins during a 20 to 30 minute period, depending on the pond size. While all observations were recorded, the present study considered only species breeding on the sampling sites. We considered as evidence of breeding the presence of eggs, larvae, or adults exhibiting reproduction-related behaviour, such as calling or mating. Larvae were captured with a handnet for identification, which was done as briefly as possible followed by the release of the individuals on the capture site. Between sampling sites all materials and footwear were sterilized using sodium hypochlorite 1% (bleach), as suggested by Speare et al. (2004) to avoid spreading possible amphibian-related diseases such as *Batrachochytrium dendrobatidis* which has caused population declines in other Iberian mountain ranges (Bosch et al., 2001; Rosa et al., 2013).

2.2.3. Environmental variable characterization

Ponds and surrounding environment were characterized using 33 variables (Table 1). Altitudinal values were taken on the field with a GPS device. Surface area, corresponding to maximum flooding capacity, was measured on the field or using satellite images (Google, DigitalGlobe) for larger ponds. Maximum depth was categorized as: (1) < 0.25 m; (2) 0.25 – 0.50 m; (3) 0.50 – 1 m; (4) 1 – 2 m; (5) > 2 m. Depending on hydroperiod ponds were classified as (1) short-term, dry before the end of April; (2) long-term, dry before the end of summer; or (3) permanent. Aquatic vegetation cover was estimated visually and categorized as: (1) < 20 %; (2) 20 – 40 %; (3) 40 – 60 %; (4) 60 - 80 %; (5) > 80 %. Watercourses were recorded as present, when a pond was connected to a stream or small river, or absent. Predatory fish, such as the introduced *Sander lucioperca* and *Carassius auratus,* were also characterized as present or absent. Local land cover composition was estimated visually within 5 and 50 m buffers around the pond shoreline, representing wetland margins and surrounding habitat, respectively. Landscape analysis (within a 500 m buffer) was made in QGIS 2.6, and was based on land-cover maps produced by the Portuguese Geographic Institute (COS2011). Road density was calculated using the same software, but since only one pond was adjacent to a road, the margin level variable was excluded. Structural diversity of land cover types, calculated with the Shannon-Wiener diversity index (Zar, 2010), for each sampling site, was used as a proxy for habitat heterogeneity at each scale.

Table 1- Variables used to characterize ponds and surrounding environment, using three distance scales, in 60 ponds in northeastern Portugal.

Variable	Code	Units	Range
Pond characteristics			
Altitude	Alt	m	(639-1403)
Area	Area	m^2	(3-2912)
Maximum depth	Depth	Score (1-5)	(1-5)
Hydroperiod	Hydro	Score (1-3)	(1-3)
Vegetation	Veg	Score (1-5)	(1-5)
Watercourse	Stream	Presence (0/1)	(0-1)
Fish	Fish	Presence (0/1)	(0-1)
Surrounding environment (within 5, 50 and 500 m)			
Bare ground	Bare5; Bare50; Bare500	%	(0-95); (0-55); (0-73)
Natural grassland	Grass5; Grass50; Grass500	%	(0-82); (0-50); (0-24)
Scrubland	Scrub5; Scrub50; Scrub500	%	(0-65); (0-77); (0-86)
Coniferous forest	Conif5; Conif50; Conif500	%	(0-28); (0-55); (0-74)
Deciduous forest	Decid5; Decid50; Decid500	%	(0-85); (0-70); (0-92)
Arable land	Agric5; Agric50; Agric500	%	(0-70); (0-70); (0-86)
Semi-natural pasture	Past5; Past50; Past500	%	(0-95); (0-55); (0-73)
Habitat heterogenity	SD5; SD50; SD500	index (continuous)	(0.20-1.69);(0.63-1.68); (0.41-1.64)
Road density	Road50; Road500	km/km^2	(0-14.49); (0-2.33)

2.2.4 Data analysis

All variables, except those classified as presence/absence, were standardized in order to minimize the effect of different measuring scales. For this study, the significance level was set as $p<0.05$ unless indicated otherwise. Analysis was made using R statistical software 3.2.1 (R Core Team, 2015). In order to test for the effects of environmental variables on species richness, we grouped variables in the following categories: pond characteristics; land cover at each buffer scale (5, 50 and 500m); habitat heterogeneity; and road density. For highly correlated variable pairs (Spearman correlation coefficient larger than ±0.6) one was retained and the other dropped. In each case, we retained the variable presenting higher correlation with species richness. We analyzed each independent variable using generalized linear modelling (GLM) with a Poisson error distribution and log-link function. Within each group, the best models were selected according to the corrected Akaike Information Criterion (AIC_c) (Burnham & Anderson, 2004). These models were then compared based on their ΔAIC_c, percentage of explained deviance relative to the null model, and on the significance of the variables.

In order to determine which variables had a relevant effect on the composition of amphibian assemblages we performed a constrained analysis of principal components (CAPC) for amphibian species incidence, using *vegan* R package (Oksanen et al., 2015). A set of environmental variables was first chosen, based on the model's AIC using a stepwise selection algorithm. The best model was then manually refined by removing non-significant variables in a stepwise approach, retesting the models at every new set of variables. We present the results for the ordination analysis based on this last set of variables.

2.3. Results

2.3.1. Species Richness

Nine of the occurring amphibian species were observed breeding in the study area. No *P.walt, P. cultripes* or *A. cisternasii* specimens were encountered and there was no evidence of breeding activity by *D. galganoi* since only one adult was found on land. Mean amphibian species richness was 2.17 ± 1.67 (SD), ranging from zero to six species per pond (Figure 2). Occupancy ranged from 2% for *B. spinosus* to 63% for *S. salamandra*. Breeding amphibians were absent in twelve ponds (20%). Species richness was best explained by hydroperiod length (

Table 2). Permanent and long-term temporary ponds, which also tended to have higher aquatic vegetation cover and maximum depth (variables excluded from the GLM analysis due to their high correlation with hydroperiod) presented more breeding species. Still at a local scale, scrubland along pond margins was positively associated with species richness, while high bare ground cover within the surrounding habitat had a negative effect. Coniferous forest cover was the only significant variable at landscape scale, with a positive correlation. Habitat heterogeneity was relevant locally, with species richness benefiting from more structurally diverse pond margins. Concerning anthropogenic impacts, no relation was found between amphibian richness and land use for agriculture or pasture at any scale. However, significant negative effects were associated with roads within 50 m from the wetlands.

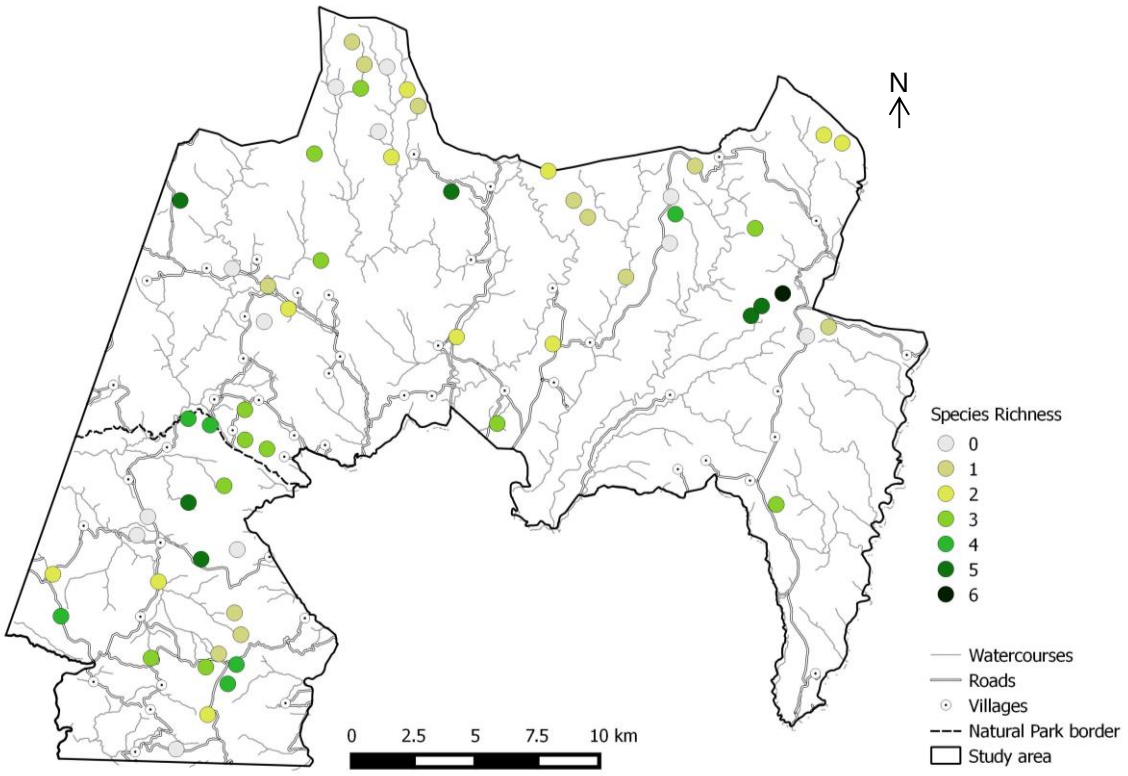

Figure 2 – Breeding amphibian species richness in 60 ponds in northeastern Portugal, between January and October 2015.

Table 2 – Independent environmental variables per category, and best generalized linear models (GLM) explaining the variance (% Dev) in amphibian species richness, in 60 ponds in northeastern Portugal.

Independent variables in category	**Best Models in category (ΔAICc<2)**	**ΔAICc**	**Estimate**	**S.E**	***p***	**% Dev**
Pond characteristics						
Alt; Area; Hydro; Stream; Fish	Hydro	0.0	0.730	0.115	<0.001	54.8
Land cover <5 m						
Bare5; Grass5; Scrub5; Conif5;Decid5; Agri5; Past5	Scrub5	44.2	0.201	0.080	0.012	6.4
Land cover <50 m						
Bare50; Grass50; Scrub50; Conif50; Decid50; Agri50; Past50	Bare50	39.2	-0.355	0.113	0.002	12.5
Land cover <500 m						
Bare500; Grass500; Scrub500; Conif500; Decid500; Agri500; Past500	Conif500	41.8	0.240	0.077	0.005	9.6
Habitat heterogeneity						
SD5; SD50; SD500	SD5	35.7	1.055	0.274	<0.001	16.5
Road density						
Road50; Road500	Road50	42.9	-0.302	0.137	0.023	7.7

2.3.2. Assemblage composition

The final constrained principal components (CAPC) model was highly significant (p<0.001) and explained 48.7 % of the variance in amphibian assemblage composition (Figure 1). Hydroperiod, water vegetation, area, fish presence, scrubland along pond margins, coniferous forest in the surrounding habitat, and grassland at a landscape scale all had significant effects (p<0.05); while presence of watercourses and deciduous forest within surrounding habitat were only marginally significant (p = 0.062; p = 0.065, respectively). Hydroperiod and water vegetation were the main components of the first CAPC axis (39.2% explained deviance), and represented a gradient of decreasing hydroperiod, vegetation, (and less importantly, area and presence of fish) in which most species incidence decreased, particularly *T. marmoratus and P. perezi*. The evident exception was *E. calamita*, which occured only in short-term temporary ponds. The second CAPC axis shows a weaker effect (9.5% explained deviance) related to a shift from landscapes with higher grassland cover, deciduous forest in the surrounding habitat and watercourse associated ponds, to habitats with more coniferous forest and higher scrubland cover in pond margins. Unlike other species, *S. salamandra* and *R. iberica* were mostly related to this axis, specifically to the former set of habitat variables.

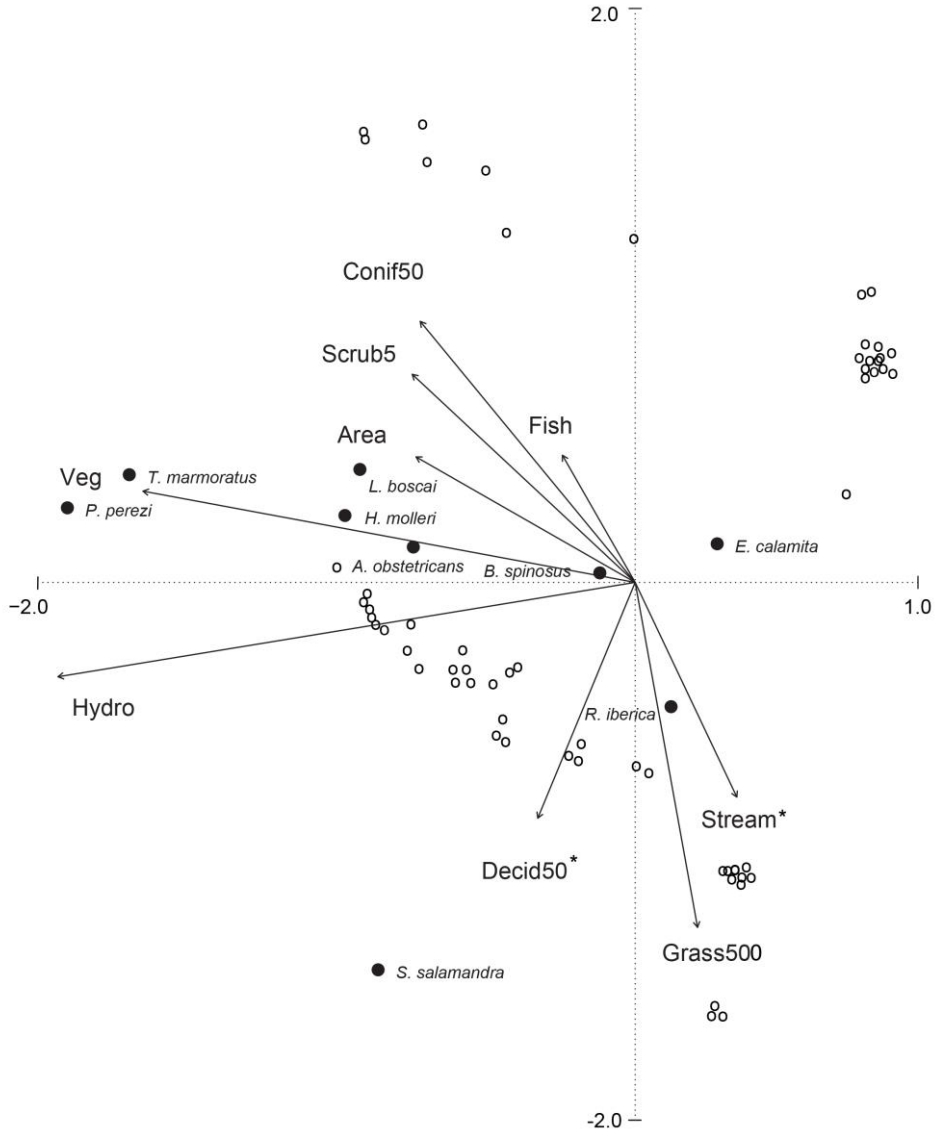

Figure 3 – Ordination bidimensional plot of the constrained analysis of principal coordinates (CAPC) for amphibian species incidence recorded in 60 ponds in northeastern Portugal, constrained by the selected set of environmental variables. Sampling sites are represented with open circles, species are black circles. Environmental gradients defined by each variable are represented by arrows; asterisks mark variables that were only marginally significant (p<0.1).

2.4. Discussion

2.4.1. Influence of wetland characteristics and land cover

Amphibian diversity was mostly influenced by local habitat factors. Pond hydroperiod was the primary factor determining species richness, and also had a strong effect on assemblage composition. As in the present study, several authors have also found hydroperiod to be have an highly important positive correlation with species richness (Pechmann et al., 1989; Snodgrass, et al., 1999; Babbitt et al., 2003). Permanent or long term temporary ponds are available for colonization for longer periods, allowing the reproduction of species with different breeding seasons, and for species with longer larval stages to complete metamorphosis (Pechmann et al.,1989). However it can negatively affect the occurrence of amphibians such as *E. calamita* which, as we also observed, has a strong preference for ephemeral temporary wetlands (Tejedo & Reques, 1992; Ferrand de Almeida et al., 2003). Longer hydroperiod also provides better conditions for aquatic vegetation to develop, another factor that shaped assemblage composition. Joly et al. (2001) and Hartel et al. (2007) also found a positive relation between anuran and newt species and aquatic vegetation, which can provide shelter, support for oviposition, food for anuran tadpoles, and perching spots for calling adults. While larger areas frequently present higher biodiversity, several studies testing this hypothesis on pond breeding amphibians failed, as we did, to find such a relation (Snodgrass et al., 1999; Oertli et al., 2002). Although pond area was associated with the presence of some species, these seemed to be better explained by hydroperiod and aquatic vegetation. Fish presence often has nefarious effects on amphibian populations due to predation of eggs, tadpoles and even adults, as many authors have documented (Hecnar & M'Closkey, 1997; Bosch et al., 2006; Hartel et al., 2007). Yet, not all species are affected, as noted by Hecnar & M'Closkey (1997). Ponds occupied by fish were rare in our sampling (8.3%), and although there seems to be a positive association with some species, overall this factor had the weakest significant effect on assemblage composition. The major effect of watercouses connected to ponds seemed to be the positive relation with *R. iberica*, a species frequently associated to mountain streams and rivers (Ferrand de Almeida et al., 2003). Even in cases where watercourse caudal decreased in late spring or summer and the connection was interrupted, tadpoles still seemed to be able to complete metamorphosis, as suggested by the high number of *R. iberica* froglets observed in later surveys of these sites.

Land cover characteristics, at a local scale, influenced both species richness and assemblage composition, most likely due to factors related with ecophysiological limits of amphibians and the necessity of shelter against predators (Wells, 2007). Within pond margins, scrubland cover appeared to be beneficial for most amphibians, although its effect on species richness was relatively weak. Still, riparian vegetation can provide protection for breeding adults (Hazell et al., 2001), while scrub in particular might provide tall cover without the negative effects of pond overshading caused by dense tree canopy (Werner & Glennemeier, 1999). Within surrounding habitat, bare ground showed the strongest effect in species richness. This effect was negative, and since the variable wasn´t important for assemblage composition, our data suggests that areas deprived of any kind of vegetation decrease amphibian diversity overall. Bare ground might act as a barrier for amphibians, due to retaining less moisture, experiencing wider temperature extremes, and providing less shelter opportunities against predators (Wells, 2007). On the other hand, neither surrounding coniferous nor deciduous forest affected species richness, but both were related to the presence of different amphibians. An association between *S. salamandra* and deciduous forest is suggested by our results, while *T. marmoratus* and *L. boscai* seem to prefer ponds near conifers, despite being more related to other factors. Forests are important terrestrial habitats for amphibians, and their proximity has often been found to influence species occurrence in ponds, especially for urudeles (Guerry & Hunter, 2002; Herrmann et al., 2005; Hartel et al., 2007). The preference of *S. salamandra* for deciduous forest was also reported by Schmidt et al., (2005) and might be that salamanders are more dependent of the cover provided by leaf litter and deadwood associated with deciduous forests (Bengtsson et al., 2000), due to the strictly terrestrial habits of the adults (unlike the two newt species).

Land cover at landscape scale was apparently less important than local factors. Nonetheless coniferous forest had positive effects on species richness, most likely due to already stated reasons, and natural grasslands were associated both with *S. salamandra* and *R. iberica*. The reason for the presence of these particular species in landscapes with grasslands doesn’t seem evident to us, however it is possible that this habitat type serves as an ecological corridor for some amphibians (Hartel et al., 2010).

2.4.2. Effects of habitat heterogeneity

Measurements of habitat heterogeneity on biodiversity, can often yield mixed results, depending on the studied subject; while structural diversity might provide more ecological niches, through wider variety of environmental conditions and resources, it can also be perceived by some taxa as habitat fragmentation, especially when anthropogenic modifications are involved (Tews et al., 2004). In our study, habitat heterogeneity along pond margins had a positive effect and was the second most important factor explaining species richness. Similar results have been reported by other authors (Canova & Marchesi, 2007; Vasconcelos et al., 2009). At such a local scale, high diversity in vegetation types can provide adequate shelter and environmental conditions (humidity, temperature) that suit different species (Parris & McCarthy, 1999). We also observed that some species responded to different habitats types depending on the scale, which suggests that heterogeneity is not only important within particular spatial scales, but also between them.

2.4.3. Anthropogenic impact and traditional farming

Road density within 50 m of breeding habitats was negatively correlated with amphibian species richness. Road impacts on amphibians are well documented in the literature (Hels & Buchwald, 2001; Argaña, et al., 2012; Garcia-Gonzalez et al., 2012), and can heavily affect species migration and dispersal through mass mortality. This confirms our perception during the sampling period and a previous report for the study area (Teixeira, 1997), in which amphibian road mortality was aggravated in mid spring, matching the breeding season of many occurring amphibians (Ferrand de Almeida et al., 2003).

On the other hand, our results suggest traditional land use has no impact on amphibian diversity, since we found no effect from both arable land and semi-natural pastures, either on species richness or assemblage composition. In this region many ponds are a valuable resource for human activities, which often require water availability throughout the year. As such, many ponds located near arable fields or pastures were associated with man-made ditches and small stony dams, which allowed them to collect and retain water for longer periods. Therefore it's also likely that traditional farming indirectly boosted amphibian species richness through the effect on hydroperiod, since water is retained for a longer period of time. In the Mediterranean context, De la Montaña

et al. (2011) found positive a positive association between amphibian species richness and certain types of both natural habitats and traditional farmland at a wider spatial scale, while the populations surveyed by Crochet et al. (2004) show remarkable stability between three decades in a forest and extensive agriculture scenario. Our results are in agreement with these studies and others done elsewhere in Europe (Hartel et al., 2010; Hartel & von Wehrden, 2013; Manenti et al., 2013), but contrast heavily with those obtained in intensive agriculture scenarios (Beja & Alcazar, 2003; García-Muñoz et al., 2010; Ferreira & Beja, 2013).

2.4.4. Conclusions and implications for conservation

The present study supports the view that traditional farming activities and biodiversity can coexist, and that these practices have important conservation value. In the face of a general habitat destruction and modification trend, such areas present increasingly rare opportunities to preserve biodiversity in the Mediterranean.

Amphibian diversity was mostly affected by local scale factors, which we believe should be the focus of conservation and habitat management strategies in similar scenarios. Wetland hydroperiod was the most important factor explaining species richness, therefore destruction or alteration of permanent and long term temporary ponds can pose the biggest threat to amphibians. Nonetheless, more ephemeral temporary ponds also need to be taken into account, as some species seem to be strictly associated with them. Destruction of vegetation, particularly within a 50 meter buffer, can also cause a decrease in amphibian species richness, due to the negative effect of bare ground areas, and the association of most species with particular types of vegetation cover. Similarly, at this scale the presence of roads can cause high mortality and act as a barrier between individuals and breeding sites. To mitigate these impacts several measures are proposed by Glista et al. (2009) including culverts, drift fences, and wildlife crossing signs; these can be employed simultaneously to maximize effects.

We believe the spatial scales used in this study were adequately representative of amphibian habitat use (Semlitsch, 2007; Daversa et al., 2012), excluding dispersal movements often carried out by juvenile individuals. Nonetheless, in areas with heavier anthropogenic disturbance, a broader spatial analysis can shed light on other important factors for amphibian diversity, especially those related with wetland connectivity (Ficetola & De Bernardi, 2004; Ribeiro et al., 2011).

CAPÍTULO 3: CONSIDERAÇÕES FINAIS

Os resultados obtidos durante este trabalho são reflexo de observações efetuadas durante uma breve janela temporal (janeiro a outubro 2015) nas comunidades de anfíbios. Estas comunidades podem apresentar marcadas diferenças interanuais na sua composição, particularmente devido ao efeito da pluviosidade nos seus habitats de reprodução (Gómez-Rodríguez et al., 2009). Assim, estes dados não devem ser interpretados como representativos de um cenário estático. De facto, a ausência de observações da atividade reprodutiva de espécies presentes na área de estudo como *P. waltl*, *A. cisternasii*, *P. cultripes*, e *D. galganoi*, poderá estar relacionada com a pluviosidade, uma vez que estas espécies se reproduzem frequentemente em massas de água temporárias (Beja & Alcazar, 2003; Ferrand de Almeida et al., 2003). Alternativamente, poder-se-á dever simplesmente à sua ocorrência rara e fragmentada na área de estudo, e possivelmente ao facto de nenhum dos charcos amostrados ser usado por estas espécies (Teixeira, 1999).

Relativamente ao trabalho de levantamento da herpetofauna realizado por José Teixeira (1997, 1998 e 1999) durante este trabalho observaram-se ocorrências em novos locais do PNM para todas as espécies amostradas (com excepção de *R. iberica*). Adicionalmente, as observações relativas à serra da Nogueira, poderão representar informação nova (considerando uma escala espacial equivalente à do levantamento de herpetofauna do PNM). A localização dos locais de amostragem, assim como as espécies encontradas em cada (Anexo 1) poderá ser assim usada para complementar o conhecimento da distribuição da herpetofauna da região. É importante salientar que todas as espécies de anfíbios observadas no PNM foram também detectadas na serra da Nogueira, às quais se junta *D. galganoi,* que foi apenas observada nesta área. Adicionalmente, os charcos da serra da Nogueira apresentaram em média mais espécies reprodutoras que os charcos do PNM (Anexo 1). Estes resultados sugerem que, ao nível da conservação de anfíbios, a Serra da Nogueira é uma área tão importante como o próprio PNM.

Para além das medidas de conservação já sugeridas, e que cuja escala espacial (<500 m) se adequa não só à conservação do habitat de anfíbios mas também ao de répteis com hábitos aquáticos (Semlitsch & Bodie, 2003), o mapeamento e caraterização das massas de água, incluindo charcos temporários, é sem dúvida essencial para a

monitorização e conservação das comunidades de anfíbios a longo prazo (Gómez-Rodríguez et al., 2009; Ribeiro et al., 2011). A criação de novos charcos, particularmente nas zonas mais áridas, é igualmente importante pois irá disponobilizar mais habitats de reprodução, favorecendo também a conectividade entre populações. No caso particular da ZPE Montesinho/Nogueira, o clima e topografia poderão também facilitar o aparecimento de doenças como a quitridiomicose. Neste sentido é fundamental o alerta para situações de mortalidade em massa junto aos corpos hídricos, assim como a aplicação de protocolos de higiene e desinfeção para o contato com estes habitats (Speare et al., 2004).

Kentwood D. Wells, no seu livro "*Ecology & Behaviour of Amphibians*" demonstra um dos problemas atuais da conservação descrevendo o seguinte episódio: "Em 1813, John James Audobon observava bandos imensos de pombos-passageiros voando sobre os bosques, em números tão grandes que "obscureciam a luz do dia como se de um eclipse se tratasse". Apesar de a população local disparar contra os pombos com todas as armas que tinha, Audubon sentia-se confiante que nenhum tipo de caça conseguiria alguma vez diminuir a imensidão destas aves. [ ] Cem anos depois, o último pombo passageiro morria solitário no jardim zoológico de Cincinnati, tendo os restantes sucumbido à caça comercial que encheu as mesas Vitorianas de tarte de pombo, mas dizimou uma espécie no processo". (Wells, 2007).

Nos dias que correm, vários anfíbios e outros seres encontram-se numa situação semelhante à do pombo-passageiro, em alguma das etapas deste episódio. Da mesma forma que vários esforços e recursos são despendidos em espécies e habitats à beira da destruição, é igualmente importante aplicar a ecologia e a conservação nas restantes situações, de forma a que estas não sejam as prioridades de amanhã.

Na opinião do autor, a *Terra Fria* transmontana conta de forma singular, histórias de convivência entre a o Homem e a Natureza. Se por um lado, algumas falam de conflitos e ideias desadequadas aos dias de hoje, muitas outras, ilustram uma filosofia de cooperação e benefício mútuo. Estas são frequentemente as mais esquecidas, e à luz de um mundo que avança com base num paradigma de industrialização e consumismo crescente, aquelas cuja conservação é mais urgente.

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Anexo I – Locais de amostragem: localização e espécies observadas.

Tabela I. 1 – Localização dos pontos de amostragem por zona, coordenadas (WGS84), hidroperíodo e espécies associadas (S – espécies encontradas no local; Sr – espécies que se reproduziram no local). Informação recolhida entre janeiro – outubro 2015.

PONTO	Zona	Coordenada N	Coordenada W	Hidroperíodo	S	Sr	Espécies
PL01	Lom	41°52'23.98"N	6°44'40.16"W	3	6	2	**Ss**, **Tm**, Lb, Ao, Bs, Pp
PL02	Lom	41°50'30.90"N	6°43'28.90"W	3	5	3	**Tm**, Ec, **Bs**, **Hm**, Pp
PL03	Lom	41°52'14.67"N	6°41'51.67"W	3	5	2	**Ss**, Tm, Lb, Bs, **Pp**
PL04	Lom	41°54'59.70"N	6°40'50.26"W	1	2	1	Lb, **Ec**
PL05	Lom	41°55'21.31"N	6°41'15.33"W	1	1	1	**Ec**
PL06	Lom	41°55'59.55"N	6°41'59.63"W	1	4	2	**Ss**, Tm, Lb, **Ec**,
PL07	Lom	41°53'39.10"N	6°39'44.75"W	3	4	1	Tm, Lb, Ao, **Ri**
PL08	Lom	41°54'25.52"N	6°38'26.40"W	1	0	0	
PL09	Lom	41°55'4.03"N	6°38'17.34"W	2	5	4	**Ss, Tm, Lb, Ao**, Pp
PL10	Lom	41°55'26.01"N	6°38'24.92"W	1	0	0	
PL11	Lom	41°56'6.24"N	6°37'43.54"W	2	4	1	**Ss**, Lb, Ec, Pp
PL12	Lom	41°56'47.89"N	6°33'57.19"W	1	4	2	**Ss**, Tm, **Lb**, Pp
PL13	Lom	41°56'35.89"N	6°33'25.66"W	2	4	2	**Ss**, Tm, **Lb**, Ri
PL14	Lom	41°54'44.98"N	6°35'58.11"W	3	4	3	**Tm, Lb**, Ao, **Pp**
PL15	Lom	41°53'19.76"N	6°35'8.56"W	3	6	6	**Ss, Tm, Lb, Ao, Hm, Pp**
PL16	Lom	41°53'4.37"N	6°35'46.13"W	3	5	5	**Ss, Tm, Ao, Hm, Pp**
PL17	Lom	41°52'51.07"N	6°36'5.81"W	3	8	5	**Ss, Tm**, Lb, **Ao**, Ec, Bs, **Hm, Pp**
PL18	Lom	41°52'24.36"N	6°34'27.54"W	1	5	0	Tm, Lb, Ao, Hm, Pp
PL19	Lom	41°52'36.87"N	6°33'49.38"W	1	7	1	**Ss**,Tm, Lb, Ao, Ec, Hm, Pp
PL20	Lom	41°48'46.28"N	6°35'21.01"W	3	6	3	**Ss**, Ao, Ec, Bs, **Hm, Pp**
PM01	Mon	41°55'33.27"N	6°44'49.51"W	3	6	5	**Ss, Tm, Lb, Ao,** Ri**, Pp**

PM02	Mon	41°57'24.26"N	6°45'47.50"W	2	5	1	**Ss**, Lb, Bs, Ri, Pp
PM03	Mon	41°57'45.39"N	6°46'6.76"W	2	6	2	**Tm**, Lb, Ao, Bs, Hm, **Pp**
PM04	Mon	41°58'14.89"N	6°46'41.61"W	1	2	0	Bs, Pp
PM05	Mon	41°58'18.37"N	6°47'21.29"W	2	4	1	Tm, Lb, Ri, **Pp**
PM06	Mon	41°58'47.67"N	6°47'42.94"W	1	2	1	**Ss**, Bs
PM07	Mon	41°57'47.08"N	6°47'27.71"W	2	4	3	**Ss, Lb, Tm**, Pp
PM08	Mon	41°57'48.75"N	6°48'11.25"W	1	2	0	Ao, Pp
PM09	Mon	41°56'50.73"N	6°46'56.83"W	1	2	0	Bs, Ri
PM10	Mon	41°56'17.84"N	6°46'33.88"W	2	2	2	**Ss, Ri**
PM11	Mon	41°56'22.68"N	6°48'48.37"W	2	6	3	**Ss, Tm, Lb**, Ec, Ri, Pp
PM12	Mon	41°54'3.27"N	6°48'37.44"W	3	6	3	**Ss,** Tm, **Lb**, Ao, Ri, **Pp**
PM13	Mon	41°55'20.94"N	6°52'43.19"W	3	6	5	**Ss, Tm, Lb**, Ao, **Hm, Pp**
PM14	Mon	41°53'52.93"N	6°51'12.62"W	2	2	0	Ri, Pp
PM15	Mon	41°53'30.33"N	6°50'9.80"W	2	6	1	**Ss**, Lb, Ao, Ec, Hm, Pp
PM16	Mon	41°53'0.66"N	6°49'33.84"W	3	5	2	**Ss**, Tm, Lb, **Ri**, Pp
PM17	Mon	41°52'43.59"N	6°50'16.38"W	1	1	0	Bs
PM18	Mon	41°50'48.96"N	6°50'50.34"W	3	4	3	**Ss, Tm**, Lb, **Pp**
PM19	Mon	41°50'9.71"N	6°50'49.93"W	3	5	3	**Ss, Tm**, Lb, Bs, **Pp**
PM20	Mon	41°49'57.99"N	6°50'11.70"W	2	4	3	**Ss, Tm**, Lb, **Pp**
PN01	Nog	41°50'37.02"N	6°52'30.10"W	3	4	4	**Ss, Tm, Lb, Pp**
PN02	Nog	41°50'28.87"N	6°51'51.34"W	3	5	4	**Tm, Lb,** Bs**, Hm, Pp**
PN03	Nog	41°49'10.01"N	6°51'26.33"W	2	5	3	**Ss, Tm,** Lb**, Hm,** Pp
PN04	Nog	41°48'47.68"N	6°52'28.27"W	3	8	5	**Ss, Tm**, Lb, **Ao**, Bs, **Hm,** Ri**, Pp**
PN05	Nog	41°47'34.15"N	6°52'7.02"W	3	7	5	**Ss, Tm**, Lb, **Ao**, Bs, **Hm, Pp**
PN06	Nog	41°47'46.56"N	6°51'1.99"W	1	0	0	
PN07	Nog	41°46'24.12"N	6°51'8.58"W	2	4	1	**Ss**, Tm, Bs, Pp
PN08	Nog	41°45'55.75"N	6°50'57.07"W	1	3	1	**Ss**, Hm, Pp
PN09	Nog	41°45'17.52"N	6°51'5.12"W	3	5	4	**Ss, Tm,** Ao**, Hm, Pp**
PN10	Nog	41°45'30.94"N	6°51'36.67"W	2	3	1	**Ss**, Bs, Pp

PN11	Nog	41°45'14.12"N	6°51'58.78"W	3	4	3	**Ss,** Tm**, Hm, Pp**
PN12	Nog	41°44'51.65"N	6°51'20.15"W	3	6	4	**Ss, Tm, Ao,** Bs, Hm**, Pp**
PN13	Nog	41°44'11.03"N	6°51'56.17"W	3	6	2	Ss**,** Tm**,** Lb**, Ao,** Bs**, Pp**
PN14	Nog	41°43'26.35"N	6°52'50.29"W	1	1	0	**Pp**
PN15	Nog	41°46'19.46"N	6°56'11.96"W	3	6	4	**Tm, Lb,** Ec, Bs**, Hm, Pp**
PN16	Nog	41°47'14.68"N	6°56'25.90"W	2	6	2	**Ss,** Tm, Lb, Dg, Bs**, Pp**
PN17	Nog	41°48'5.32"N	6°53'59.18"W	1	1	0	Bs
PN18	Nog	41°48'29.84"N	6°53'40.43"W	1	2	0	Tm, Pp
PN19	Nog	41°47'5.10"N	6°53'22.60"W	2	4	2	**Ss,** Ao, Bs**, Ri**
PN20	Nog	41°45'25.28"N	6°53'34.41"W	3	5	3	**Ss, Tm,** Lb**,** Bs**, Pp**

Zona: Lom = Lombada; Mon = Montesinho; Nog = Nogueira;

Nota: A divisão da área de estudo em diferentes zonas deveu-se a razões de organização logística. A zona “Montesinho” engloba charcos que não se encontram nesta serra.

Hidroperíodo: 1 = temporário curto; 2 = temporário longo; 3 = permanente

Espécies: Ss = Salamandra salamandra; Tm = Triturus marmoratus; Lb = Lissotriton boscai; Ao = Alytes obstetricans; Dg = Discoglossus galganoi; Ec = Epidalea calamita; Bs = Bufo spinosus; Hm = Hyla molleri, Ri = Rana iberica; Pp = Pelophylax perezi

Nota: Espécies reprodutoras a negrito.

Média, desvio-padrão, e amplitude da riqueza específica de anfíbios reprodutores dos charcos do PNM e da serra da Nogueira:

- **PNM = 2.1 ± 1.64 (0-6)** (Lombada = 2.2 ± 1.74 (0-6); Montesinho = 1.9 ± 1.59 (0-5))
- **Nogueira = 2.4 ± 1.73 (0-5)**

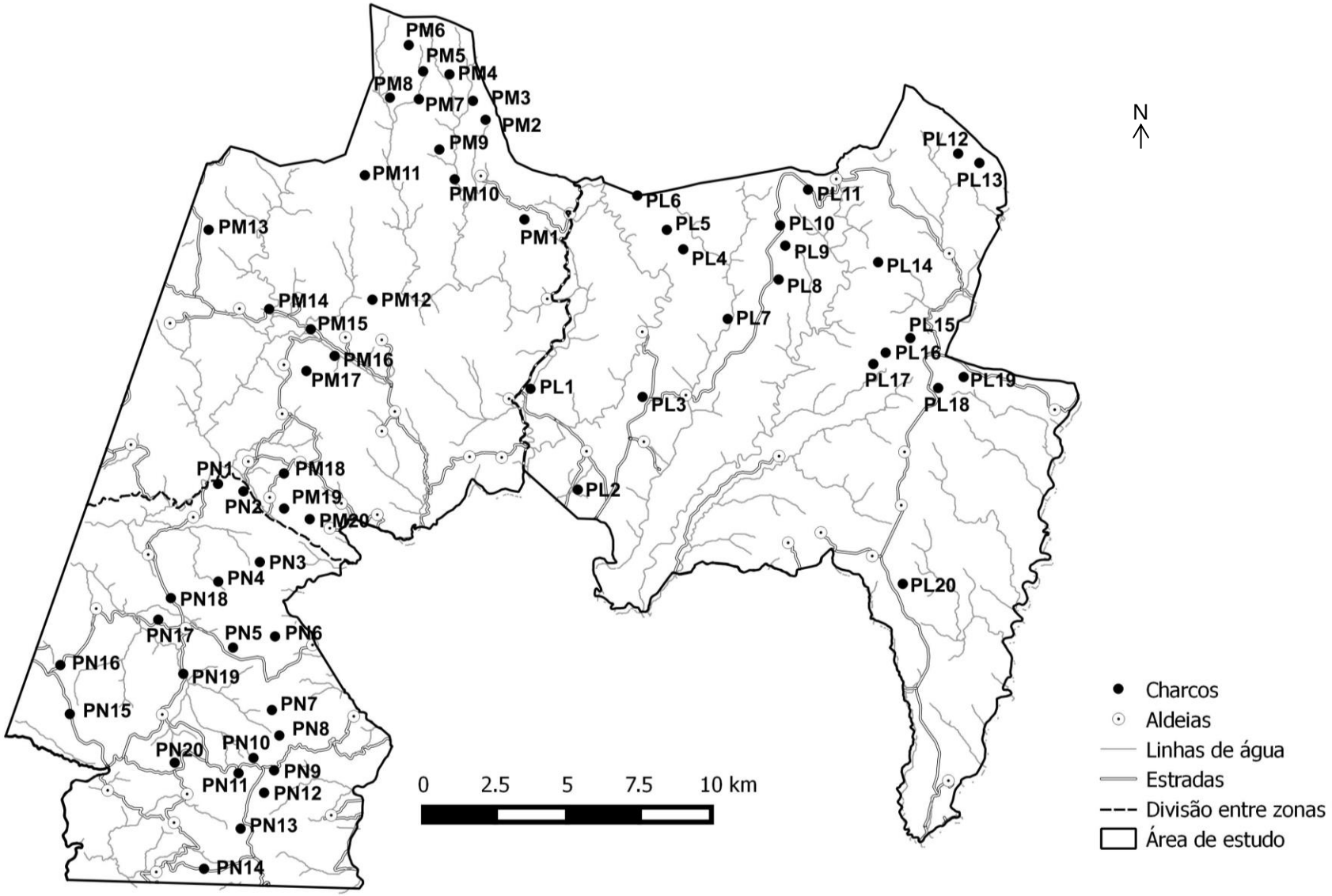

Figura I. 1 – Localização dos charcos amostrados no Parque Natural do Montesinho e serra da Nogueira, nordeste de Portugal, entre janeiro e outubro 2015.

Anexo II – Fotografia: área de estudo, habitats, e anfíbios.

Figura II.1 – Serra do Montesinho, em janeiro 2015.

Figura II. 2 – Campos junto ao rio Baceiro, em abril 2015.

Figura II. 3 – Solo nú.

Figura II. 4 – Herbáceas naturais.

Figura II. 5 – Matos.

Figura II. 6 – Floresta de coníferas

Figura II. 7 – Floresta de folhosas.

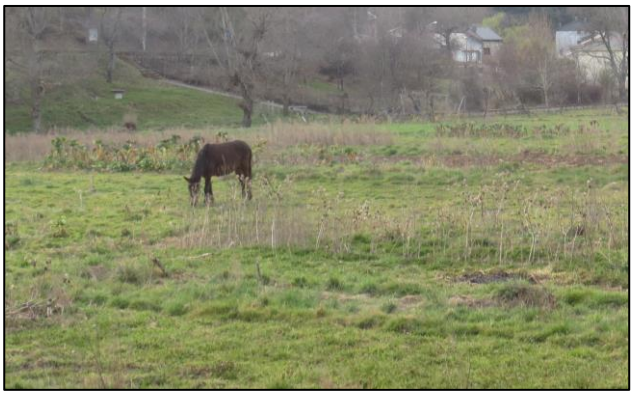

Figura II. 8 – Campos agrícolas.

Figura II. 9 – Pastagem (lameiro).

Figura II. 10 – Charco permanente.

Figura II. 11 – Charco temporário de longa duração.

Figura II. 12 – Charco temporário de curta duração.

Figura II. 13 – Amostragem noturna em fevereiro 2015.

Figura II. 14 – Rã iberica (*Rana iberica*).

Figura II. 15 – Larva de tritão-marmorado (*Triturus marmoratus*).

Figura II. 16 – Amplexo de rã-verde (*Pelophylax perezi*).

Figura II. 17– Metamorfo de rela-comum (*Hyla molleri*).

Figura II. 18 – Reprodução de sapo-parteiro-comum (*Alytes obstetricans*).

Anexo III – Modelos para a riqueza específica.

Table III. 2 – Generalized linear models (GLMs) tested for each independent variable, explaining the variance (% Dev) in amphibian species richness, in 60 ponds in northeastern Portugal.

Independent variables in category	ΔAICc	Estimate	S.E	*p*	% Dev
Pond characteristics					
Alt	50.1	-0.091	0.092	0.323	1.1%
Area	46.2	0.164	0.068	0.017	5.3%
Hydro*	0.0	0.730	0.115	<0.001	54.8%
Stream	49.8	-0.236	0.211	0.262	1.4%
Fish	50.9	0.112	0.303	0.711	0.2%
Land cover <5 m					
Bare5	4.7	-0.099	0.094	0.291	1.3%
Grass5	3.3	-0.153	0.098	0.121	2.8%
Scrub5*	0.0	0.201	0.080	0.012	6.4%
Conif5	5.7	-0.037	0.095	0.700	0.2%
Decid5	5.8	-0.016	0.090	0.854	0.1%
Agric5	5.8	0.010	0.086	0.904	0.0%
Past5	5.3	0.063	0.084	0.454	0.6%
Land cover <50 m					
Bare50*	0.0	-0.355	0.113	0.002	12.5%
Grass50	7.5	-0.197	0.102	0.054	4.4%
Scrub50	11.2	-0.054	0.091	0.555	0.4%
Conif50	4.7	0.201	0.072	0.005	7.4%
Decid50	11.2	0.051	0.086	0.554	0.4%
Agric50	11.5	0.022	0.086	0.801	0.1%
Past50	10.9	0.071	0.084	0.398	0.8%
Land cover <500 m					
Bare500	5.2	-0.209	0.120	0.082	4.0%
Grass500	8.9	-0.027	0.092	0.771	0.1%
Scrub500	5.4	-0.171	0.092	0.063	3.8%
Conif500*	0.0	0.240	0.077	0.002	9.6%
Decid500	8.9	-0.016	0.090	0.860	0.1%
Agric500	8.6	0.054	0.085	0.521	0.5%
Past500	8.2	-0.086	0.100	0.391	0.9%
Habitat heterogeneity					
SD5*	0.0	1.055	0.274	<0.001	16.5%
SD50	8.6	0.897	0.389	0.011	7.3%
SD500	13.4	0.444	0.316	0.160	2.2%
Road density					
Road50*	0.0	-0.302	0.130	0.020	7.7%
Road500	7.2	0.008	0.088	0.925	0.0%

* Best models selected in each category based on their ΔAIC_c